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Root-Shoot Interaction in the Greening of Wheat Seedlings Grown under Red Light[1]

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Wheat seedlings grown with roots exposed to constant red light (300–500 μmol m^{-2} s^{-1}) did not accumulate chlorophyll in the leaves. In contrast, seedlings grown with their roots shielded from light accumulated chlorophylls. Chlorophyll biosynthesis could be induced in red-light-grown chlorophyll-deficient yellow plants by either reducing the red-light intensity at the root surface to 100 μmol m^{-2} s^{-1} or supplementing with 6% blue light. The inhibition of chlorophyll biosynthesis was due to impairment of the Mg-chelatase enzyme working at the origin of the Mg-tetrapyrrole pathway. The root-perceived photomorphogenic inhibition of shoot greening demonstrates root-shoot interaction in the greening process.

Gallium-aluminum-arsenide LEDs, with high output in the red region of photosynthetic absorption and action spectra, offer a tremendous technical advantage over conventional light sources for plant growth in space (Bula et al., 1991). The advantages of LEDs over other light sources are long life, small weight and volume, and the solid state nature of the device. The spectral output of red LEDs used in the present investigation has a peak wavelength at 660 nm and a bandwidth of 26 nm. This peak wavelength corresponds to the peak of photosynthetic action spectrum of plants (McCree, 1972). There are substantial differences in the spectral distribution of light in various plant habitats, and plants tend to adapt the structure of photosynthetic apparatus and pigment composition to light quality (Smith, 1975; Buschmann et al., 1978; Senger, 1980; Leong and Anderson, 1984; Eskins et al., 1985; Kim et al., 1993; Kaufman, 1993). To evaluate the suitability of LEDs in photosynthesis and developmental processes, wheat (*Triticum aestivum* L. cv Yecora Rojo) plants were grown under continuous illumination of various intensities from red LEDs and were compared with plants grown under white (cool-white fluorescent plus incandescent) light regimes.

MATERIALS AND METHODS

Plant Growth

Wheat (*Triticum aestivum* L. cv Yecora Rojo) seedlings were grown for 7 d under white-light (cool-white fluorescent plus incandescent) or red-light (gallium-aluminum-arsenide LEDs; Quantum Devices Inc., Barneveld, WI) regimes (100 or 500 μmol m^{-2} s^{-1}). Two germination regimes were used. In the first, plants were grown on moistened germination paper (Seedburo Equipment Co., Chicago, IL) in Petri plates, which allowed the roots to be exposed to light. There was no increase in temperature at the root surface when plants were grown on germination paper. In the second, plants were grown in Petri dishes in vermiculite (A.H. Hummert Seed Co., St. Louis, MO) and had their roots covered and shielded from light. The seedlings were grown at 25°C and 60% RH and were watered with nutrient solution (Wheeler et al., 1991). When required, red light was supplemented with 10 and 25 μmol m^{-2} s^{-1} of blue fluorescent light (F20712/BB, 20 W; Philips Lighting Co, Somerset, NJ).

Biosynthesis of Plant Pigments

For the determination of biochemical lesion(s) of Chl biosynthetic reactions, intermediate tetrapyrroles of the Chl biosynthetic pathway were estimated. Five batches of 100 mg of excised leaves were incubated in the dark for 4 h with 5 mM ALA, the precursor of tetrapyrroles. After incubation the leaves were homogenized in 90% ammoniacal acetone in the dark, and Proto IX, MP(E), and Pchlide were estimated from the pigment extract by spectrofluorometry. To determine the rate of Pchlide synthesis from the endogenous substrates and their subsequent phototransformation to Chlide by the enzyme Pchlide reductase, 10 batches of 100 mg of excised leaves were floated in water (in the absence of ALA) in the dark for 4 h. Five of 10 batches of leaf material incubated in the dark were homogenized in 90% ammoniacal acetone in the dark and their Pchlide pools synthesized from endogenous substrates were analyzed by spectrofluorometry. The remaining five batches of leaf material, after 4 h of dark incubation, were exposed to cool-white fluorescent light (200 μmol m^{-2} s^{-1}) for 2 min to phototransform synthesized Pchlide to Chlide and were immediately homogenized, and the residual level of Pchlide was determined by spectrofluorometry. Chl was determined according to the method of Arnon (1949).

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Abbreviations: ALA, 5-aminolevulinic acid; LED, light-emitting diode; MP(E), Mg-protoporphyrin IX and its monoester; Proto IX, protoporphyrin IX.

Spectrofluorometry

Hexane was added to the acetone extract. Fully esterified tetrapyrrole Chl was extracted in hexane, whereas carboxylic tetrapyrroles Proto IX, MP(E), and Pchlide remained in the aqueous hexane-extracted acetone residue solvent mixture (Tripathy and Rebeiz, 1988). Quantitative estimation of Proto IX, MP(E), and Pchlide thus partitioned was carried out spectrofluorometrically as described previously (Rebeiz et al., 1975; Hukmani and Tripathy, 1992). The fluorescence spectra of the hexane-extracted acetone residue solvent mixture were recorded in the ratio mode using an SLM (Urbana, IL) Aminco 8000 C spectrofluorometer. The spectra were corrected for the photomultiplier tube sensitivity. Rhodamine B was used in the reference channel as a quantum counter. The photomultiplier tube was cooled to −20°C to increase the signal to noise ratio. The emission spectra were recorded from 580 to 700 nm at excitation and emission bandwidths of 4 nm.

RESULTS AND DISCUSSION

Seedlings grown with covered roots under 100 or 500 μmol m^{-2} s^{-1} of red or white lights synthesized and accumulated Chl and underwent greening. Seedlings grown with their roots exposed to white light (100 or 500 μmol m^{-2} s^{-1}) or red light (100 μmol m^{-2} s^{-1}) also accumulated Chl. However, plants germinated and grown with their roots exposed under higher photon fluence rates of red light (500 μmol m^{-2} s^{-1}) did not accumulate Chl and looked yellow for 5 d and turned white after 7 to 10 d (Fig. 1). Using white seed germination paper instead of brown

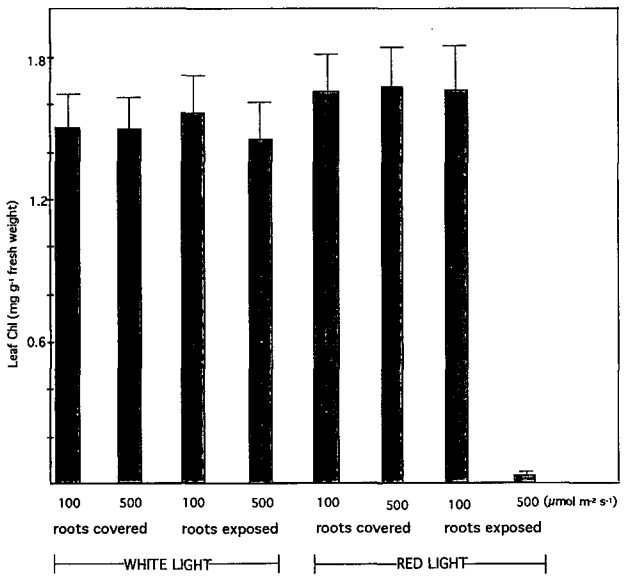

Figure 1. Accumulation of Chl in wheat seedlings grown for 7 d under white-light (cool-white fluorescent plus incandescent) or red-light (gallium-aluminum-arsenide LEDs) regimes (100 or 500 μmol m^{-2} s^{-1}). The seedlings were germinated and grown on germination paper in Petri dishes or in vermiculite at 25°C and 60% RH and were watered with nutrient solution as described in "Materials and Methods." The vertical bars represent SD.

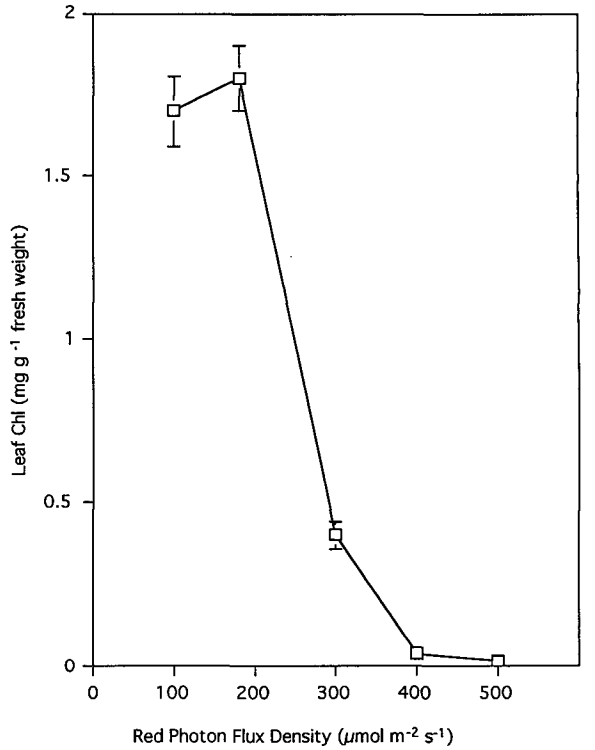

Figure 2. The light-response curve of Chl accumulation by the seedlings grown on germination paper with roots exposed to red light. Wheat seedlings were grown under different intensities of red light. The vertical bars represent SD. The missing error bars indicate that they are smaller than the label marks.

also resulted in yellow Chl-deficient seedlings grown under red light (500 μmol m^{-2} s^{-1}). Quartz sand was used to create a rooting environment similar to the vermiculite treatment with the exception that some red light penetrated to the root surface. The result was the same, i.e. yellow Chl-deficient seedlings (data not shown).

To ascertain the amount of light that inhibits greening, seedlings were grown on germination paper under different light intensities of red light and the amounts of Chl synthesized by the leaves were estimated. The light-response curve of Chl accumulation revealed that Chl biosynthesis was inhibited by 80% at 300 μmol m^{-2} s^{-1} and was almost abolished at 400 to 500 μmol m^{-2} s^{-1} (Fig. 2).

To probe the role of roots in this response, the wheat seedlings were germinated in the dark in Petri dishes either on germination paper or in vermiculite for 5 d and were transferred to 100 and 500 μmol m^{-2} s^{-1} of red or white light for 48 h. Etiolated seedlings grown in vermiculite accumulated Chl when exposed to red or white light at both light intensities. However, the plants that had roots exposed, i.e. grown on germination paper, when exposed to 500 μmol m^{-2} s^{-1} of red light failed to produce Chl, although they underwent greening and accumulated Chl under 100 μmol m^{-2} s^{-1} of red light or both of the intensities of white light (Fig. 3). These experiments suggest that the roots play a significant role in the greening process.

In a follow-up test, 40 seedlings were grown on germination paper under continuous red-light illumination (500

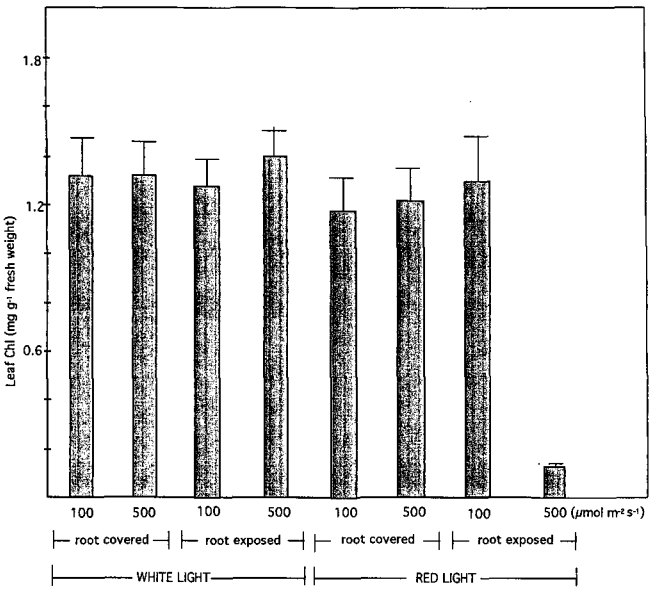

Figure 3. Greening of 5-d-old etiolated wheat seedlings grown on germination paper or in vermiculite under white- or red-light regimes (100 or 500 μmol m^{-2} s^{-1}). The vertical bars represent SD.

μmol m^{-2} s^{-1}) for 5 d and these lacked Chl. If the roots of these seedlings were subsequently covered with vermiculite and grown for an additional 4 d under 100 or 500 μmol m^{-2} s^{-1} of red or white light, 80% of the plants produced Chl and green shoots (Table I). Reducing the red-light intensity at the root surface by transferring the plants to 100 μmol m^{-2} s^{-1} red light or exposing them to white light (100 or 500 μmol m^{-2} s^{-1}) also induced Chl biosynthesis in 70 to 80% of the plants within 4 d. Those seedlings that had their roots exposed and remained under 500 μmol m^{-2} s^{-1} of red light for the additional 4 d were completely white. However, leaves of seedlings grown under red light for a longer duration (10 d) when transferred to white light (500 μmol m^{-2} s^{-1}) did not turn green. Only the new leaves produced after the transfer to white light synthesized Chl.

These experiments demonstrate that the lack of Chl accumulation by the leaves is due to the exposure of roots to relatively higher amounts of red light and it could be reversed by reducing the red-light intensity or transferring the plants to white light. In contrast, when white-light-grown 5-d-old green plants with exposed roots were transferred to high-intensity red light (500 μmol m^{-2} s^{-1}) for 14 d, they did not turn white but remained green (data not shown). This demonstrates that the root-perceived red-light-induced inhibition of Chl biosynthesis is manifested during early seedling growth and that red light cannot reverse greening once the process is initiated.

Blue light, a component of the white light spectrum, is known to play a significant role in gene expression and photomorphogenesis (Senger, 1980; Reymond et al., 1992; Kaufman, 1993; Short et al., 1993). To probe the role of blue light, seedlings were grown with roots exposed to 400 μmol m^{-2} s^{-1} red light supplemented with 25 μmol m^{-2} s^{-1} blue fluorescent light. Blue-light-supplemented seedlings accumulated Chl to the same extent as those grown with roots shielded from a high intensity (400 μmol m^{-2} s^{-1}) of red light (Fig. 4). Reducing the amount of blue light to 10 μmol m^{-2} s^{-1} reduced the amount of Chl in the leaves by 70%.

To identify the biochemical lesion(s) that inhibit the Chl biosynthetic reactions (Rebeiz et al., 1983; Tripathy and Rebeiz, 1986; Beale and Weinstein, 1990; Leeper, 1991; Richards, 1993), green and Chl-deficient white seedlings were grown with their roots exposed to white or red light (500 μmol m^{-2} s^{-1}) for 5 d. Leaves were then excised for pigment analysis. Although α,α'-dipyridyl is known to

Table I. *Greening of red-light-grown white seedlings by exposure of the roots to different intensities of red or white light*

Wheat seedlings were grown on germination paper in Petri dishes under continuous illumination of red light (500 μmol m^{-2} s^{-1}) at 25°C and 60% RH and were watered with half-strength nutrient solution. After 5 d the roots either remained exposed or were covered with vermiculite and were grown for an additional 4 d under 100 or 500 μmol m^{-2} s^{-1} of red or white lights. After the treatment the white and green seedlings were counted.

Root Exposure Status	Light Quality	Light Intensity	White Plants	Green Plants	Greening
		μmol m^{-2} s^{-1}	*No. of plants*		%
Exposed	Red	500	40	0	0
Exposed	Red	100	8	32	80
Exposed	White	500	10	30	75
Exposed	White	100	12	28	70
Covered	Red	500	11	29	72
Covered	Red	100	9	31	77
Covered	White	500	8	32	80
Covered	White	100	10	30	75

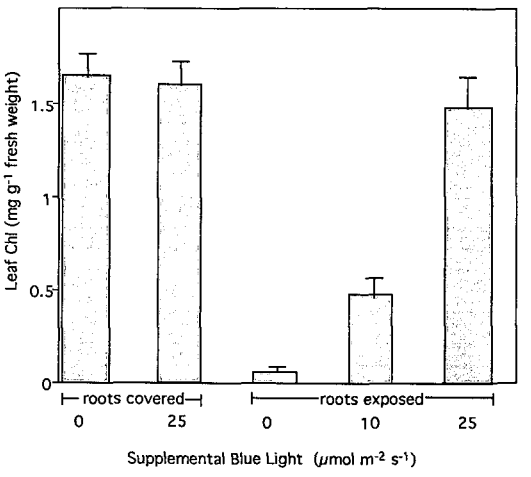

Figure 4. Greening of wheat seedlings grown under continuous illumination of 400 μmol m^{-2} s^{-1} of red light supplemented with 0, 10, and 25 μmol m^{-2} s^{-1} of blue fluorescent light. Wheat seedlings were grown on germination paper with their roots exposed to light or in vermiculite with their roots covered and shielded from light. The seedlings were grown as described in "Materials and Methods." The vertical bars represent SD.

stimulate the production of Mg-tetrapyrroles (Vlcek and Gassman, 1979), we found that ALA, the precursor of tetrapyrroles, was sufficient to produce Mg-protoporphyrins in wheat leaves. Therefore, the excised leaves were incubated in the dark with ALA, and pigments were determined. Seedlings grown under red light synthesized 52% less tetrapyrroles [consisting of Proto IX, MP(E), and Pchlide] than the white-light-grown plants (Fig. 5A). Proto IX was the predominant form of tetrapyrrole (84–86% of total tetrapyrrole) that accumulated in red-light-grown plants, whereas Pchlide (63% of total tetrapyrrole) was the most abundant Chl precursor synthesized in white-light-grown plants. In the latter, Proto IX constituted only 8% of tetrapyrrole produced in response to ALA treatment. Compared to white-light-grown plants, synthesis of MP(E) was highly inhibited by 93 to 95% in red-light-grown plants.

Consequently, the synthesis of Pchlide, which is formed from the MP(E), was diminished by 91 to 94%. This demonstrates that in the leaves of the high-intensity red-light-grown plants the Mg-tetrapyrrole pathway leading to Chl biosynthesis was inhibited at its origin, i.e. at the step of Mg^{2+} insertion to Proto IX mediated by Mg-chelatase enzyme. Tissue elemental analysis of red-light-grown plants revealed that they were not deficient in Mg. The ability to synthesize tetrapyrroles from the endogenous substrates was investigated by incubating the leaves in dark in the absence of ALA, and the tetrapyrroles were quantified by spectrofluorometry. Only trace amounts of Proto IX, MP(E), and Pchlide were detected in excised leaves of seedlings grown with roots exposed to red light (500 μmol m^{-2} s^{-1}). The ability to synthesize Pchlide declined by 90%, and when the excised leaves were exposed to white light (200 μmol m^{-2} s^{-1}) for 2 min, 90 to 95% of Pchlide

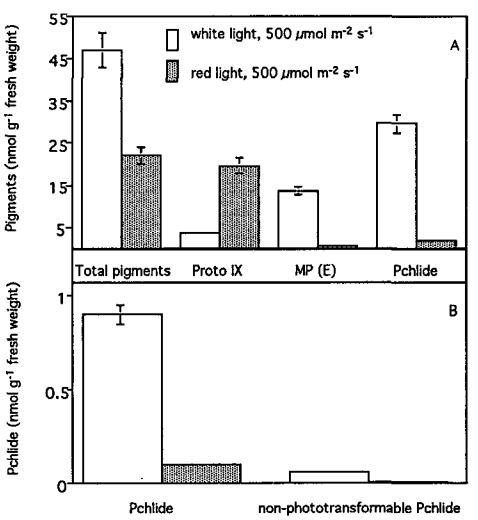

Figure 5. A, Synthesis of Proto IX, MP(E), and Pchlide in response to ALA treatment; B, phototransformation of Pchlide synthesized from endogenous substrates in excised leaf discs of wheat seedlings grown on germination paper with exposed roots under 500 μmol m^{-2} s^{-1} of white or red light. The pigments were estimated by spectrofluorometry as described in "Materials and Methods." The vertical bars represent SD. The missing error bars indicate that they are smaller than the label marks.

phototransformed, leaving 5 to 10% as nonphototransformable Pchlide (Fig. 5B), suggesting that the activity of Pchlide reductase was not affected in the red-light-grown plants.

These results demonstrate the root-shoot interaction in the greening process. The root-perceived photomorphogenic inhibition of Chl biosynthesis in the shoots of wheat seedlings by moderately high photon fluence density of red light is caused by the impairment of the Mg-chelatase enzyme working at the origin of the Mg-tetrapyrrole pathway and is manifested during early seedling growth. It will be highly interesting to investigate the chemical nature of root photoreceptors involved in perceiving the red light and the molecular mechanism of inhibition of Mg-chelatase in the leaves by the exposure of roots to red light.

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